PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 38/30, 9/00, 9/14

A1

(11) International Publication Number: **WO 99/55362**

(43) International Publication Date: 4 November 1999 (04.11.99)

(21) International Application Number: PCT/US99/09077

(22) International Filing Date: 27 April 1999 (27.04.99)

(30) Priority Data:
09/069,684 29 April 1998 (29.04.98) US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: SPRAY DRIED FORMULATIONS OF IGF–I

(57) Abstract

The invention relates generally to spray–dried dry powder compositions of IGF–I, which may or may not contain excipients.

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SPRAY DRIED FORMULATIONS OF IGF-1

FIELD OF THE INVENTION

The invention is directed to a spray-dried IGF-I formulation, in particular, to a formulation without excipients.

BACKGROUND OF THE INVENTION

Human insulin-like growth factor-I (IGF-I) is a homolog of proinsulin, and is a single-chain 7649-dalton polypeptide with a pI of 8.4 with three disulfide bonds. Rinderknecht and Humbel, Proc. Natl. Acad. Sci. USA, 73: 2365 (1976); Rinderknecht and Humbel, J. Biol. Chem., 253: 2769 (1978)); Daughaday and Rotwein, Endocr. Rev., 10: 68-91 (1989). It belongs to a family of somatomedins with insulin-like and mitogenic biological activities. IGF-I appears to mediate most of the metabolic actions of growth hormone (GH) *in vivo*. Salmon and Daughaday, J. Lab. Clin. Med., 49: 825-836 (1957); Schelechter *et al.*, Proc. Natl. Acad Sci. USA, 83: 7932-7934 (1986); Van Wyk *et al.*, Recent Prog. Horm. Res., 30: 259 (1974); Binoux, Ann. Endocrinol., 41: 157 (1980); Clemmons and Van Wyk, Handbook Exp. Pharmacol., 57: 161 (1981); Baxter, Adv. Clin. Chem., 25: 49 (1986); U.S. Pat. No. 4,988,675; WO 91/03253; and WO 93/23071. One notable exception is its effects on fatty acid and glucose metabolism. Mauras and Haymond, Pediatr. Nephrol., 10: 318-323 (1996).

Because of its protein anabolic effect (Clemmons *et al.*, J. Clin. Endocrinol. Metab., 75: 234-238 (1992); Mauras and Beaufrere, J. Clin. Endocrinol. Metab., 80: 869-874 (1995); Fryburg, Am. J. Physiol., 267: E331-E336 (1994); Horber and Haymond, J. Clin. Invest., 86: 265-272 (1990)), its glucose lowering effect (Guler *et al.*, N. Engl. J. Med., 317: 137-140 (1987); Turkalj *et al.*, J. Clin. Endocrinol. Metab., 75: 1186-1191 (1992)), and its growth-promoting effects (Schoenle *et al.*, Nature, 296: 252-253 (1982); Tomas *et al.*, J. Endocrinol., 137: 413-421 (1993)), this peptide is being investigated in a multiplicity of clinical settings. These include attempts to preserve lean body mass in critically ill patients (Clemmons *et al.*, *supra*; Miell *et al.*, Clin. Endocrinol., 37: 542-551 (1992); Lieberman *et al.*, J. Clin. Endocrinol. Metab., 78: 404-410 (1994); Koea *et al.*, Endocrinology, 131: 643-648 (1992)); to improve the metabolic status of diabetics (Schoenle *et al.*, Diabetologia, 34: 675-679 (1991); Kuzuya *et al.*, Diabetes, 42: 696-705 (1993); Zenobi *et al.*, J. Clin. Invest., 90:2234-2241 (1992); Non-Insulin-Dependent Diabetes Study Group (RINDS), Diabetes, 45: (abstract #55) (1996)); and to promote growth in patients with GH-receptor deficiency. Wilton, Acta Paediatr. Suppl., 383: 137-142 (1992); Rosenbloom *et al.*, Trends Endocrinol. Metab., 5: 296-303 (1994). Other encouraging results have been reported in acute and chronic renal failure (Hirschberg *et al.*, Kidney Int., 43: 387-397 (1993); Miller *et al.*, Kidney Int., 46: 201-207 (1994); Guler *et al.*, Acta Endocrinol., 121: 101-106 (1989); Guler *et al.*, Proc. Natl. Acad. Sci. USA, 86: 2868-2872 (1989); U.S. Pat. No. 5,106,832), in the treatment of osteoporosis (Rosen *et al.*, Proc. Soc. Exp. Bio. Med., 206: 83-102 (1994); McCarthy *et al.*, Connect Tissue Research, 20: 277-282 (1989); Bagi *et al.*, Bone, 16: 595-665 (1995)), and even in neuromuscular diseases (Lewis *et al.*, Exp. Neurol., 124: 73-88 (1993); Barinaga, Science, 264: 772-774 (1994)) and brain trauma. Johnston *et al.*, J. Clin. Invest., 97: 300-308 (1996).

Various methods of treating patients using IGF-I, including its use in the treatment of obesity, are disclosed in the patent literature, including U.S. Pat. Nos. 5,273,961; 5,597,797; 5,126,324; 5,187,151; 5,202,119; 5,374,620; 5,106,832; 4,988,675; 5,106,832; 5,068,224; 5,093,317; and 4,876,242; WO 92/11865; and WO 94/16722.

Further, it was found that in GH-sufficient postabsorptive individuals, the metabolic effects of rhIGF-I are in part dependent on the mode of administration, with a robust protein-anabolic effect when rhIGF-I is given as twice daily bolus injections but no detectable effect on protein turnover after a continuous mode of delivery. Mauras *et al.*, Am. J. Physiol., 272: E628-E633 (1997).

Researchers have found that recombinant human insulin-like growth factor-1 (rhIGF-1) can improve glycemic control and enhance insulin sensitivity in patients with a syndrome of severe insulin resistance. Schoenle *et al.*, Diabetologia, 34: 675-679 (1991); Usala *et al.*, N. Eng. J. Med., 327: 853-857 (1992); Morrow *et al.*, J. Clin. Endocrinol. Metab., 79: 205-210 (1994). In addition, rhIGF-I was also found to improve glucose and lipid metabolism in patients with noninsulin-dependent (Type II) diabetes mellitis. Zenobi *et al.*, J. Clin. Invest., 90: 2234-2241 (1992); Schalch *et al.*, J. Clin. Endocrinol. Metab., 77 (6) 1563-1568 (1993); Moses *et al.*, Diabetes, 45: 91-100 (1996). Although the efficacy and mechanism of action of rhIGF-I in the regulation of insulin and blood glucose levels in these patients are still unclear, the protein may be considered as an alternative therapy in the treatment of diabetes when the patients become insensitive to insulin treatment.

However, rhIGF-I therapy currently requires several daily injections, which is undesirable from a patient perspective and raises concerns about patient compliance. Thus, other delivery routes, specifically pulmonary administration via inhalation delivery, would be desirable.

Inhalation therapy involves the administration of a drug in an aerosol form to the respiratory tract. Aerosol delivery is based on the concept that delivery to the deep lung regions (alveoli), which account of 95% of lung epithelia, can significantly enhance the transport of the protein through the epithelial membrane if the molecule is bioavailable.

Two general types of aerosols are employed: liquid particles and solid particles. The liquid aerosols are generated by nebulizing solutions of the drug. Solid particles are either in the form a powder suspended in a propellant which is administered from a metered dose inhaler or simply as a powder that is administered from a dry powder inhaler. In the case

of polypeptide drugs, solid particle aerosols are typically made by lyophilizing (also known as freeze-drying) the drug from solution and then milling or grinding the lyophilized drug to the desired particle size distribution for pulmonary administration.

Recently, the possibility of using spray-drying to formulate aerosol powders of therapeutic proteins has been discussed. Spray drying is a dehydration process that utilizes heat from a hot gas stream (usually air) to evaporate dispersed droplets created by atomization of a continuous liquid feed. Using these methods, products can be dried within a few seconds into fine particles, and this general process has been used for decades to prepare dry pigments and dairy powders. As applied to specific therapeutic proteins, however, thermal denaturation and structural alterations are a concern. This is generally attributed to the loss of hydration water molecules required to form hydrogen bonds to stabilizes the secondary structure; generally spray-drying is done with excipients such as carbohydrates that can act as water-replacing agents.

There are several reports of spray drying therapeutic proteins for pulmonary delivery. Maa et al. report on the use of polysorbate-20 surfactant to form stable, rhGH formulations. J. Pharm. Sci. 87(2):152 (1998). See also Mumenthaler et al., Pharm. Res. 11(1):12 (1994); Chan et al., Pharm. Res. 14(2):431 (1997), and WO 97/41833, which discusses the spray-drying of biological macromolecules.

Thus, there is a need to provide new spray-dried formulations of IGF-I suitable for inhalation therapy, i.e. pulmonary administration.

SUMMARY OF THE INVENTION

Accordingly, the present invention provides dry powder compositions of spray-dried insulin-like growth factor I (IGF-I) suitable for pulmonary administration comprising particles of IGF-I of an average size 2 to 4 μm. Preferred powders are substantially free of an excipient.

In a further aspect the invention provides methods of preparing the compositions comprising spray-drying an aqueous mixture of IGF-I under conditions to provide a respirable dry powder.

In an additional aspect, the present invention provides methods for aerosolizing a spray-dried IGF-I dispersible dry powder composition comprising dispersing an amount of the dry powder in a gas stream to form an aerosol. The aerosol may be captured in a chamber suitable for subsequent inhalation by a patient.

In a further aspect, the invention provides methods of administering a therapeutically effective dose of IGF-I to a patient comprising administering to the alveolar regions of the lungs of said patient a dry powder composition of the invention.

In an additional aspect, the invention provides methods of treating an IGF-I associated disorder comprising administering to the alveolar regions of the lungs of a patient a dry powder composition of the invention.

In a further aspect, the invention provides unit dosage receptacles and dry powder inhalers comprising a therapeutically effective amount of the dry powder compositions of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A, 1B, 1C, 1D and 1E show scanning micrographs of spray dried powders of (a) formulation A, (b) formulation E, (c) formulation K, (d) formulation D, and (e) formulation J.

Figures 2A, 2B, 2C and 2D depict scanning micrographs of blends of (a) formulation C, (b) formulation F, (c) formulation G and (d) formulation H.

Figures 3A and 3B depict scanning micrographs of blend of formulation F after 4-week storage at 2-8°C.

Figure 4 shows the effect of storage condition on spray-dried arginine-containing rhIGF-I (formulation F) after 4-week storage at 2-8°C. Size distribution of spray-dried rhIGF-I upon inhalation.

Figure 5 depicts a schematic representation of a multiple-stage liquid impinger system.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to methods and compositions comprising spray-dried formulations of IGF-I, that show good dispersibility and respirable properties, as well as good stability. In a further aspect, the present invention is directed to the surprising discovery that a stable dry powder formulation of IGF-I can be prepared without the use of excipients, i.e. protectants. Generally, as is known in the art, spray dry formulations of proteinaceous drugs require the use of excipients such as carbohydrates, polyamino acids, surfactants, etc., both for stability during the spray drying process and for shelf stability. However, surprisingly, IGF-I is able to form stable and highly useful dry powders for aerosol pulmonary administration in the absence of such excipients.

The success of a dry powder inhalation product is based on the ease of powder dispersibility, which is mainly determined by the efficiency of inhalation devices, the composition of the formulation and by the physical properties of the powder. Many physical characteristics affect the dispersibility of the powder, including the nature of the material, particle size/distribution, particle shape/morphology, and moisture content (Hickey et al., 1994). All these properties affect the interparticle (cohesion) forces and/or the particle-surface (adhesion) forces. Increased interparticle cohesion reduces powder segregation, that is, increases powder aggregation, resulting in physically larger particles that are difficult to inhale into the deep lung. Increased particle-surface adhesion decreases powder flowability and increases powder retention on all contact surfaces, ultimately resulting in less delivery to the lung.

Accordingly, the present invention provides a spray-dried insulin-like growth factor I (IGF-I) dry powder composition. By "spray dried" herein is meant that the composition is

prepared by spray drying. Spray drying is a process in which a homogeneous aqueous mixture of IGF-I, termed herein the "pre-spray dried IGF-I formulation", is introduced via a nozzle (e.g. a two-fluid nozzle), spinning disk or an equivalent device into a hot gas stream to atomize the liquid formulation to form fine droplets. The liquid formulation is preferably a solution, although suspensions, slurries or the like may be used as long as it is homogeneous to ensure uniform distribution of the IGF-I in the solution and ultimately in the powdered composition. The liquid formulations are preferably aqueous, although organic solvents may be used as well. The water or solvent rapidly evaporates from the droplets producing a fine dry powder having particles of a specified size and characteristics, as are more fully discussed below. Suitable spray drying methodologies are also described below.

By "insulin-like growth factor I" or "IGF-I" refers to insulin-like growth factor from any species, including bovine, ovine, porcine, equine, and preferably human, in native-sequence or in variant form, and from any source, whether natural, synthetic, or recombinant. Recombinant human insulin-like growth factor I (rhIGF-I) is a single chain small protein of 70 amino acids. There are three disulfide bonds in the molecule. Two of the disulfide bonds were known to isomerize in certain conditions and resulted in a IGF-I mixture containing the authentic and misfolded form. Upon long term storage, some solutions (mostly in neutral or alkaline solutions), of the authentic and the misfolded IGF-I forms can reach a equilibrium ratio of 3 to 1. IGF-I is a very basic protein (pI = 8.65), and thus it's solubility improves at lower pH. Additionally, the disulfide isomerization usually is minimized at low pHs. Misfolded IGF-I is not bioactive, its formation is the major stability consideration for the development of IGF-I formulations. The misfolded form can be detected and monitored using a reversed-phase HPLC.

Preferred herein for animal use is that form of IGF-I from the particular species being treated, such as porcine IGF-I to treat pigs, ovine IGF-I to treat sheep, bovine IGF-I to treat cattle, *etc.* Preferred herein for human use is human native-sequence, mature IGF-I, more preferably without a N-terminal methionine, prepared, *e.g.*, by the process described in EP 230,869 published August 5, 1987; EP 128,733 published December 19, 1984; or EP 288,451 published October 26, 1988. More preferably, this native-sequence IGF-I is

recombinantly produced and is available from Genentech, Inc., South San Francisco, CA for clinical investigations.

The preferred IGF-I variants are those described in U.S. Pat. Nos. 5,077,276; 5,164,370; or 5,470,828; or in WO 87/01038, *i.e.*, those wherein at least the glutamic acid residue is absent at position 3 from the N-terminus of the mature molecule or those having a deletion of up to five amino acids at the N-terminus. The most preferred variant has the first three amino acids from the N-terminus deleted (variously designated as brain IGF, tIGF-I, des(1-3)-IGF-I, or des-IGF-I).

The term "powder" means a composition that consists of finely dispersed solid particles that are relatively free flowing and capable of being readily dispersed in an inhalation device and subsequently inhaled by a patient so that the particles can reach the alveoli of the lung. Thus, the powder is "respirable" and suitable for pulmonary delivery.

The term "dispersibility" means the degree to which a powder composition can be dispersed (i.e. suspended) in a current of air so that the dispersed particles can be respired or inhaled into the lungs of a subject. Thus, a powder that is only 20% dispersible means that only 20% of the mass of particles can be suspended for inhalation into the lungs.

The average particle size and the range of particle sizes are very important in the characterization of the powders of the invention. Preferably the average particle size is less than about 5 microns (μm) in diameter with a relatively uniform spheroidal shape distribution. More preferably, the particles are less than about 4 μm, with less than about 3-4 μm being particularly preferred. Using the techniques of the invention, the general particle size distribution is between about 1 μm and about 25 μm, preferably between about 1 and about 10, with an average particle size of about 2-4 μm. The average particle size of the powder can be measured as mass mean diameter (MMF) by conventional techniques.

In addition to the average particle size, the size distribution of the particles of the powder is also important. In a preferred embodiment, the powders of the invention are

characterized on the basis of their fine particle fraction (FPF). The FPF is a measure of the aerosol performance of a powder, with the higher the fraction, the better. The FPF is defined as powder with an aerodynamic mass median diameter of less than 6.8 μm as determined using a multiple-stage liquid impinger with a glass throat (MLSI, Astra, Copley Instrument, Nottingham, UK) through a dry powder inhaler (Dryhaler, Dura Pharmaceuticals); see Figure 5. Accordingly, the IGF-I spray-dried powders of the invention preferably have a FPF of at least about 10%, with at least about 25% being preferred and at least about 35% being especially preferred, with some systems enabling very high FPFs, in the order of 50 to 70%.

The term "dry" means that the composition has a moisture content such that the particles are readily dispersible in an inhalation device to form an aerosol. This moisture content is generally below about 15% by weight water, with less than about 10% being preferred and less than about 5% being particularly preferred.

In addition, the powders of the invention comprise substantially bioactive IGF-I. That is, as is known for many dry powder formulations, some percentage of the protein in the powder can aggregate, resulting in a loss of activity. Similarly, there may be misfolded protein present, that may or may not aggregate. Accordingly, preferred embodiments provide powders that have at least about 70% active IGF-I (i.e. the percentage of active IGF-I to total IGF-I present), with at least about 80% active IGF-I being preferred, and at least about 90% active IGF-I being especially preferred. The total IGF-I present is measured by absorption at A_{276} using an extinction coefficient of 0.645 ml/(mg·cm). Active IGF-I is measured either using a known bioassay or the reverse phase HPLC system detailed in the examples, which allows a determination of correctly folded IGF-I which is assumed to be active.

In one embodiment, the spray-dried IGF-I compositions of the invention may contain excipients. "Excipients" or "protectants" generally refer to compounds or materials that are added to ensure or increase the stability of the protein during the spray-dry process. As this is done at elevated temperatures it has generally been assumed that carriers or excipients are required. Suitable excipients are basically innocuous when inhaled by a

patient and do not significantly interact with the IGF-I in a manner that alters its biological activity. Suitable excipients include, but are not limited to, proteins such as human and bovine serum albumin, gelatin, and immunoglobulins, carbohydrates including monosaccharides (galactose, D-mannose, sorbose, etc.), disaccharides (lactose, trehalose, sucrose, etc.), cyclodextrins, and polysaccharides (raffinose, maltodextrins, dextrans, etc.); an amino acid such as monosodium glutamate, glycine, alanine, arginine or histidine, as well as hydrophobic amino acids (tryptophan, tyrosine, leucine, phenylalanine, etc.); a methylamine such as betaine; an excipient salt such as magnesium sulfate; a polyol such as trihydric or higher sugar alcohols, *e.g.* glycerin, erythritol, glycerol, arabitol, xylitol, sorbitol, and mannitol; propylene glycol; polyethylene glycol; Pluronics; and combinations thereof. Preferred excipients are carbohydrates, including trehalose, mannitol, sucrose, lactose and sorbitol. Generally, when excipients are used, they are used in concentrations ranging from about 1 to 95 wt %, with from about 1 to 50 wt % preferred, and from about 1 to 20 wt % being especially preferred.

In a preferred embodiment, the spray-dried IGF-I compositions of the invention do not contain substantial amounts of excipients, i.e. they are substantially free of excipients. "Substantially free" in this case generally means that the composition contains less than about 10%, with less than about 5%, and preferably less than 2-3 % by weight any components other than IGF-I and the residual water. Generally, for the purposes of this invention, excipients do not include solvents, buffers or salts. Thus, preferred embodiments utilize spray dry formulations (prior to the addition of bulking agent, discussed below) that consist of IGF-I as the major component, with small amounts of buffers, salts and residual water.

In a preferred embodiment, the pre-spray dry formulations (i.e. the solution formulation used in the spray dry process) comprise IGF-I in water, with only negligible amounts of buffers or other compounds present. As is outlined in the Examples, pre-spray dry formulations comprising IGF-I in water are not highly stable over long periods, and thus it is desirable to perform the spray-drying process within a reasonable short time after producing the pre-spray dry formulation. However, while the pre-spray dry formulations comprising IGF-I in water are not highly stable, the dry powders made from these

formulations are both surprisingly stable and highly dispersible, as shown in the Examples.

In a preferred embodiment, the formulations that are spray dried to form the compositions of the invention comprise IGF-I in buffer, which may or may not additionally contain some salts. As is known in the art, IGF-I can be stable at acidic pH, and thus buffers active in the acidic pH range are preferred. Also, in general, pharmaceutically acceptable buffers are preferred. Thus, preferred pH ranges of the pre-spray dry formulation are from about 1 to about 8, with from about 2 to about 7 being particularly preferred, and from about 2 to about 6.5 being especially preferred. Buffers at about pH 3.0 are the most preferred. As will be appreciated by those in the art, there are a large number of suitable buffers that may be used. Suitable buffers include, but are not limited to, sodium acetate, sodium citrate, sodium succinate, ammonium bicarbonate and carbonate. Generally, buffers are used at molarities from about 1 mM to about 2 M, with from about 2 mM to about 1 M being preferred, and from about 10 mM to about 0.5 M being especially preferred, and 50 to 200 mM being particularly preferred.

In a preferred embodiment, the formulations that are spray dried to form the compositions of the invention comprise IGF-I in solvents, which may or may not additionally contain some salts. As is known in the art, IGF-I can be stable at acidic pH, and thus acidic solvents are preferred, with pharmaceutically acceptable solvents preferred. Suitable pH and molarity ranges are as outlined above for buffers. As will be appreciated by those in the art, there are a large number of suitable solvents that may be used. Suitable solvents include, but are not limited to, acids including acetic and citric acid, and alcohols such as ethanol.

In addition, when water, buffers or solvents are used, they may additionally contain salts. Generally, salts are used at molarities from about 1 mM to about 2 M, with from about 2 mM to about 1 M being preferred, and from about 10 mM to about 0.5 M being especially preferred, and 50 to 200 mM being particularly preferred. Suitable salts include, but are not limited to, NaCl.

Preferred pre-spray dry formulations include water, 100 mM acetic acid, pH 3.0 and 100 mM acetic acid, 100 mM NaCl, pH 3.0.

In some cases, the drying process appears to remove solvents or buffers, particularly volatile solvents such as acetic acid, resulting in negligible amounts of solvent present in the dry powder formulation.

In addition, the compositions of the invention are generally substantially free of "penetration enhancers". "Penetration enhancers" are surface active compounds that promote penetration of a drug through a mucosal membrane or lining and are generally used intranasally, intrarectally, and intravaginally. The use of penetration enhancers in the lungs however, is generally undesirable as the sensitive and fragile epithelial blood barrier in the lung can be severely affected by surface active compounds such as detergents. The dry powder compositions of the invention are readily absorbed in the lungs without the need to employ penetration enhancers.

Furthermore, the powders of the invention are preferably stable. "Stability" can mean one of two things, with preferred embodiments showing stability in both areas. In a preferred embodiment, stability refers to the retention of IGF-I biological activity. In a preferred embodiment, stability can also refer to the retention of dispersibility of a formulation over time.

In a preferred embodiment, the dry powders of the invention retain biological activity over time, i.e. retains its physical and chemical stability and integrity upon storage. Various analytical techniques for measuring IGF-I stability are available in the art and are reviewed in *Peptide and IGF-I Drug Delivery,* 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, New York, Pubs. (1991) and Jones, A. *Adv. Drug Delivery Rev.* 10: 29-90 (1993). The biological assay for IGF-I measures the incorporation of tritiated thymidine into cells as a measure of cell proliferation and is known in the art. Losses in biological activity are generally due to protein aggregation, misfolding or oxidation. As will be appreciated by those in the art, there may be an initial loss of biological activity as a result of spray-drying, due to the high temperatures used in the process. However, once this has occurred,

further loss of activity should be minimized; that is, stability in this context is measured from the time the powder is made, rather than before the powder is made.

In a preferred embodiment, the dry powders of the invention retain dispersibility over time. Generally, this is quantified by the retention of a high FPF over time; that is, the powder minimally aggregates, cakes or clumps over time.

Stability can be measured at a selected temperature for a selected time period. As will be appreciated by those in the art, the length of time and the conditions under which a formulation should be stable will depend on a number of factors, including the amount made per batch, the storage conditions (temperature, relative humidity, etc.), the turnover of the product, etc. Generally, for rapid screening, a matrix of conditions are run. Commonly, formulations may be tested at 2-8°C, 30°C and sometimes 40°C, for periods of 2, 4 and 24 weeks. These tests are usually done at 38% relative humidity (rh), as is outlined in the Examples. Thus, in a preferred embodiment, the powders of the invention preferably lose less than about 20-30% of their biological activity over 18 months, with losses of less than about 10% being preferred and less than about 5% being especially preferred. Similarly, when dispersibility is being evaluated, the powders of the invention lose less than about 50% of their FPF, with losses of less than about 25% being preferred and losses of less than about 20% being especially preferred.

In a preferred embodiment, the IGF-I powders of the invention are later combined with bulking agents or carriers as is known in the art, which are used to reduce the IGF-I concentration in the powder being delivered to a patient; that is, it may be desirable to have larger volumes of material per unit dose. Bulking agents may also be used to improve the dispersibility of the powder within a dispersion device, and/or to improve the handling characteristics of the powder. This is distinguishable from the use of bulking agents or carriers during the spray drying process. Suitable bulking agents are generally crystalline (to avoid water absorption) and include, but are not limited to, lactose and mannitol. Accordingly, bulking agents such as lactose, if added, may be added in varying ratios, with from about 99:1 rhIGF-I to bulking agent to about 1:99 being preferred, and

from about 1:5 to about 5:1 being more preferred, and from about 1:10 to about 1:20 being especially preferred.

In one embodiment, the powders of the invention are formulated with other drugs. For example, the powders of the invention may be formulated with hypoglycemic agents. The term "hypoglycemic agent" refers to compounds that are useful for regulating glucose metabolism. More preferred herein for human use are insulin and the sulfonylurea class of oral hypoglycemic agents, which cause the secretion of insulin by the pancreas. Examples include glyburide, glipizide, and gliclazide. In addition, agents that enhance insulin sensitivity or are insulin sensitizing, such as biguanides (including metformin and phenformin) and thiazolidenediones such as REZULIN™ (troglitazone) brand insulin-sensitizing agent, and other compounds that bind to the peroxisome proliferator activated receptor (PPAR) subtype PPARγ nuclear receptor, or that activate RXR, are within this definition, and also are preferred. For additional examples of PPARγ and RXR activators, see WO 97/10813 and WO 97/10819. Thus preferred embodiments utilize IGF-I co-formulation with insulin or human growth hormone.

The compositions of the invention may also comprising preservatives, detergents, surfactants, antioxidants, etc., as will be generally known in the art.

The compositions of the invention are generally made as follows. Generally, IGF-I is made recombinantly as is known in the art. It may be formulated for stability as a liquid formulation in any number of formulations. Generally, for spray-drying, the liquid formulations are subjected to diafiltration and ultrafiltration, as required, for buffer exchange (or removal, in the case of IGF-I in water) and/or concentration, as is known in the art. Generally, the pre-spray dry formulations comprise from about 5 mg/ml to about 75 mg/ml of IGF-I, with from about 10 mg/ml or about 60 mg/ml being preferred, and from about 20 to about 60 mg/ml being especially preferred. The use of buffers and excipients, if present, are done at concentrations discussed above.

The pre-spray dry formulation is then spray dried, as is generally known in the art. See for example Maa et al. supra, Mumenthaler et al., supra, Chan et al., supra, and WO 97/41833,

and WO 95/31479 , all of which are expressly incorporated herein by reference. Generally, the pre-spray dry formulation is atomized as is known in the art, for example via a two-fluid nozzle using filtered pressurized air, into a stream of hot gas, usually air, although nitrogen and inert gases may also be used. The atomization conditions, including atomization gas flow rate, atomization gas pressure, liquid flow rate, etc., are generally controlled to produce liquid droplets having an average diameter of from about 5 to about 30 μm, with droplets of average size 10 μm being preferred. Conventional spray drying equipment is generally used, such as Büchi, Niro Yamato, Okawara, Kakoki and the like.

The temperature of the inlet of the gas used to dry the sprayed IGF-I can range, as will be appreciated by those in the art, and can depend on the composition, including the presence or absence of excipients. Generally, the inlet temperature ranges from about 70 to about 180 °C, with from about 80 to about 150°C being preferred, and from about 90 to about 120°C being especially preferred.

The outlet of the gas used for drying the powder can also range. Generally, the outlet temperatures range from about 40 to about 150 °C, with from about 50 to about 120°C being preferred, and from about 50 to about 80°C being especially preferred.

In addition, it may be desirable to have additional heating, i.e. a secondary drying step, at the same or different temperatures, after spray drying is complete. Thus, for example, it may be desirable to leave the powder at an elevated temperature for some period of time to reduce the moisture content to the desired level. This is generally done under vacuum at temperatures ranging from 30 to 50°C, with 40°C being preferred.

The powders are collected using conventional techniques, and bulking agents, if desirable, are added, and the powders may be loaded into unit dosages and/or dry powder inhalers.

Once made, the powders of the invention are capable of being readily dispersed by an inhalation device and subsequently inhaled by a patient so that the particles are able to penetrate into the alveolar regions of the lungs of the patient. Thus, the powders of the invention are formulated into unit dosages comprising therapeutically effective amounts of

IGF-I, and used to deliver IGF-I to a patient, for example for the treatment of IGF-I associated disorders.

The IGF-I to be used in the therapy will be formulated and dosed in a fashion consistent with good medical practice, taking into account, for example, the type of disorder being treated, the clinical condition of the individual patient (especially the side effects of treatment with IGF-I), whether the IGF-I is administered for preventative or therapeutic purposes, the concentration of the IGF-I in the dosage, previous therapy, the patient's clinical history and response to the IGF-I, the method of administration, the scheduling of administration, the discretion of the attending physician, and other factors known to practitioners. The incidence of side effects of IGF-I may be reduced by decreasing the dose. Kupfer *et al.*, J. Clin. Invest., 91: 391-396 (1993); Hartman *et al.*, J. Clin. Invest., 91: 2453-2462 (1993). The "effective amount" or "therapeutically effective amount" of IGF-I for purposes herein is thus determined by such considerations and is an amount that increases and maintains the relevant, favorable biological response of the mammal. Generally, a therapeutically effective amount of IGF-I ranges from about 5 μg/kg twice per day to 150 μg/kg twice per day. The IGF-I is suitably administered to the patient at one time or over a series of treatments and may be administered to the patient at any time from diagnosis onwards.

Thus, the present invention provides spray-dried dry powder formulations of IGF-I in unit dosages. A "unit dosage" as discussed herein means a unit dosage receptacle containing a therapeutically effective amount of IGF-I. The dosage receptacle is one that fits within a suitable inhalation device to allow for the aerosolization of the IGF-I powder formulation by dispersion into a gas stream to form an aerosol. These can be capsules, foil pouches, vials, etc. The container may be formed from any number of different materials, including plastic, glass, foil, etc. The container generally holds the spray-dried powder, and includes directions for use. The unit dosage containers may be associated with inhalers that will deliver the powder to the patient. These inhalers may optionally have chambers into which the powder is dispersed, suitable for inhalation by a patient.

Additionally, the powder compositions of the invention may be further formulated in other ways, for example, in the preparation of sustained release compositions, for example for implants, patches, etc. This may be done using polymers, as is known in the art. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, *e.g.*, films, or microcapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman *et al.*, Biopolymers, 22, 547-556 [1983]), poly(2-hydroxyethyl methacrylate) (Langer *et al.*, J. Biomed. Mater. Res., 15: 167-277 (1981), and Langer, Chem. Tech., 12: 98-105 [1982]), ethylene vinyl acetate (Langer *et al.*, *supra*) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). The spray-dried powder can also be used to prepare a PROLEASE™ formulation of IGF-I. Sustained-release IGF-I compositions also include liposomally entrapped IGF-I. Liposomes containing IGF-I are prepared by methods known *per se*: DE 3,218,121; Epstein *et al.*, Proc. Natl. Acad. Sci. U.S.A., 82: 3688-3692 (1985); Hwang *et al.*, Proc. Natl. Acad. Sci. U.S.A., 77: 4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appln. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (from or about 200 to 800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal therapy.

The dry powders of the invention may also be used for dry powder injection. In this embodiment, the dispersibility and respirability of the powder is not important, and the particle size may be larger, for example in the 20 to 70 μm range.

It should also be noted that the powder compositions of the invention may be reconstituted for injection as well. That is, the powders of the invention show good stability, and thus in some embodiments they can be reconstituted into liquid form using a diluent and used in non-pulmonary routes of administration, for example, via injection (subcutaneously, intraveneously, etc.). In this embodiment, any number of known diluents can be used, as will be appreciated by those in the art, including physiological saline, other buffers, salts, etc. Alternatively, it is also possible to reconstitute the powder and use it to form liquid aerosols for pulmonary delivery.

In another embodiment, the invention provides lyophilized IGF-I that is substantially free of excipients. That is, the liquid formulations of the invention that do not utilize excipients can be used in traditional lyophilization techniques, the results of which may be milled or ground to result in respirable, dispersible dry powders for inhalation.

The compositions of the invention are useful to deliver IGF-I to a patient, for example for the treatment of a IGF-I associated disorders. By "IGF-I associated disorder" or "disease state responsive to treatment by IGF-I" or "a disorder requiring treatment with IGF-I" is any condition that would benefit from treatment with IGF-I, including but not limited to, for example, diabetes, lung diseases, hyperglycemic disorders as set forth below, renal disorders, such as acute and chronic renal insufficiency, end-stage chronic renal failure, glomerulonephritis, interstitial nephritis, pyelonephritis, glomerulosclerosis, *e.g.*, Kimmelstiel-Wilson in diabetic patients and kidney failure after kidney transplantation, obesity, GH-insufficiency, Turner's syndrome, Laron's syndrome, short stature, undesirable symptoms associated with aging such as obesity and increased fat mass-to-lean ratios, immunological disorders such as immunodeficiencies including decreased CD4 counts and decreased immune tolerance or chemotherapy-induced tissue damage, bone marrow transplantation, diseases or insufficiencies of cardiac structure or function such as heart disfunctions and congestive heart failure, neuronal, neurological, or neuromuscular disorders, *e.g.*, peripheral neuropathy, multiple sclerosis, muscular dystrophy, or myotonic dystrophy, and catabolic states associated with wasting caused by any condition, including, *e.g.*, trauma or wounding or infection such as with a bacterium or human virus such as HIV, wounds, skin disorders, gut structure and function that need restoration, and so forth. The disorder being treated may be a combination of two or more of the above disorders. The preferred disorders targeted for treatment herein are hyperglycemic disorders, including diabetes, obesity, heart disfunctions, kidney disorders, neurological disorders, whole body growth disorders, and immunological disorders. As used herein, the term "hyperglycemic disorders" refers to all forms of diabetes and disorders resulting from insulin resistance, such as Type I and Type II diabetes, as well as severe insulin resistance, hyperinsulinemia, and hyperlipidemia, *e.g.*, obese subjects, and insulin-resistant diabetes, such as Mendenhall's Syndrome, Werner Syndrome, leprechaunism, lipoatrophic diabetes, and other lipoatrophies. The preferred

hyperglycemic disorder is diabetes, especially Type 1 and Type II diabetes. "Diabetes" itself refers to a progressive disease of carbohydrate metabolism involving inadequate production or utilization of insulin and is characterized by hyperglycemia and glycosuria.

As used herein, the term "treating" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those prone to having the disorder or diagnosed with the disorder or those in which the disorder is to be prevented. Consecutive treatment or administration refers to treatment on at least a daily basis without interruption in treatment by one or more days. Intermittent treatment or administration, or treatment or administration in an intermittent fashion, refers to treatment that is not consecutive, but rather cyclic in nature. The treatment regime herein can be consecutive or intermittent or any other suitable mode. In addition, the term "treating" includes management of a particular disorder, as in the management of hyperglycemic disorders and obesity.

The following examples serve to more fully describe the manner of using the above-described invention, as well as to set forth the best modes contemplated for carrying out various aspects of the invention. It is understood that these examples in no way serve to limit the true scope of this invention, but rather are presented for illustrative purposes. All references cited herein are incorporated by reference in their entirety.

EXAMPLES

Evaluation of Dry Powder Formulations

Preparation of recombinant human insulin-like growth factor I:

Varying amounts of carbohydrates (trehalose or mannitol) and amino acids (histidine and/or L-arginine) were used to prepare inhalation formulations as follows:

(A) pure rhIGF-I, pH 7.0;

(B) rhIGF-I, 100 mM acetic acid, pH 3.0;

(C) rhIGF-I, 100 mM acetic acid, 100 mM sodium chloride, pH 3.0;

(D) Injection formulation (see below);

(E) rhIGF-I, 10 mM histidine, pH 5.5;

(F) rhIGF-I, 230 mM L-arginine, pH 7.4;

(G) rhIGF-I, 10 mM histidine, 230 mM L-arginine, pH 7.3;

(H) rhIGF-I:trehalose @ 80:20 (weight ratio), 10 mM histidine, pH 5.5;

(I) rhIGF-I:trehalose @ 60:40 (weight ratio), 10 mM histidine, pH 5.5;

(J) rhIGF-I:mannitol @ 80:20 (weight ratio), 10 mM histidine, pH 5.5;

(K) rhIGF-I:trehalose @ 60:40 (weight ratio), 10 mM histidine and 230 mM L-arginine, pH 5.5;

(L) rhIGF-I:trehalose @ 60:40 (weight ratio), 10 mM histidine and 230 mM L-arginine pH 7.4.

Recombinant humanized insulin-like growth factor (rhIGF-I), a molecular weight of 7,648 Dalton, was produced in two different formulations. The first formulation (used as D, above) is formulated for injection and contains 10 mg/ml IGF-I in 5.84 mg/ml NaCl, 9 mg/ml benzyl alcohol, 2 mg/ml polysorbate 20, and 50 mM sodium acetate, pH 5.4. The second formulation is similar, and contains 10 mg/ml of IGF-1 and 9 mg/ml benzyl alcohol, 10 mM histidine, and 230 mM arginine, pH 7.3. The formulation was modified by increasing IGF-I concentration to 31 mg/ml and eliminating the benzyl alcohol in the formulation, this high IGF-I in-process liquid bulk with 10 mM histidine, and 230 mM arginine, pH 7.3 was designated as the his-arg formulation (G, above).

The his-arg formulation was prepared from the protein pooled from S-Sepharose, the step prior to the formulation of IGF-I into its final acetate formulation. In the S-Sepharose step, IGF-I was eluted in 200 mM Sodium Citrate, pH 6.0; the pooled IGF-I, in general, has a high protein concentration. The S-Sepharose pool that we used in this report has a IGF-I concentration of 29.9 mg/ml. To eliminate the citrate in the S-Sepharose pool, IGF-I solution was buffer exchanged by the tangential flow filtration (ultrafiltration (UF)/diafiltration (DF) using Millipore Pellicon regenerated cellulose membrane (5KPLCC-C) with a 5,000K molecular weight cut-off. The citrate in the S-Sepharose pooled liquid was first replaced by NaCl using the his-arg formulation plus 100 mM NaCl. The NaCl in this preparation was then eliminated by a second tangential flow filtration using the his-arg buffer . After buffer exchange, IGF-I was concentrated to 31 mg/ml and

sterile filtered. The his-arg formulation plus 80:20 and 60:40 of IGF-I : trehalose, respectively, were also prepared as in-process liquids for IGF-I dry powder process.

All formulations were prepared using ultrafiltration/diafiltration, followed by dialysis for buffer exchange into various pre-spray dry formulations and/or the additions of excipients. Mannitol and trehalose were used.

Some UF/DF runs were performed in a fully automated TFF system. Details of the system has been described elsewhere (see van Reis et al., Biotechnol. Bioeng. 55:737-746 (1997), hereby incorporated by reference).

Pre-spray dry formulation preparation and stability

In-process bulk liquid formulation is very important for the preparation of protein dry powders. The non-volatile components will be present in the powders, therefore affects the powder appearance and the chemical stability and physical properties of the resulting powder. To minimize manufacturing cost and increase facility capacity, formulation candidates should offer high protein solubility to reduce liquid volume and dry powder process time. To maximize production schedule flexibility, an in process liquid formulation that is stable for at least 30 days at 2 - 8°C is desirable. A desirable in-process liquid formulation also needs to offer good chemical stability and fine powder properties after spray drying for protein dry powder preparation

Preparation of pre-spray dry formulations:

Four general types of in-process liquid formulations were prepared and processed using the above methods for IGF-I aerosol dry powders: 1) formulations derived from IGF-I subcutaneous injection products (D, G, L above), 2) histidine-containing formulations, with or without the addition of trehalose (E, G, H, I, J and K), 3) excipient free aerosol powder formulations (A and B), and 4) simple formulations (C and F).

Stability of pre-spray dry formulations

The appearance of IGF-I in process bulk formulations was examined visually. The first examination was performed immediately after dialysis and IGF-I was in the dialysis

cassettes. The second examination was performed after the liquids were transferred into sterilized 30 ml glass vials. These liquid were sterile filtered and then examined again. pH, volume, and protein concentration of in-process liquid formulations were measured. The protein concentration was monitored by UV spectroscopy at A_{268}. Percentage of protein recovery were calculated to monitor the loss of IGF-I during the buffer exchange process for the preparation of IGF-I in-process bulks. IGF concentration in the liquids was also determined by SEC-HPLC.

IGF-I in-process liquids prepared in water and acetic acid were examined immediately after buffer exchange in the dialysis cassettes and were examined again after the liquids were transferred into 30 ml glass vials. The acetic acid in-process bulk formulation was clear. However, IGF-I concentration was reduced slightly upon buffer exchange of the original his-arg formulation to the acetic acid formulation due to a slight increase in volume. The resulted IGF-I concentration in the acetic acid formulation was 28.3 mg/ml and the pH of the solution was 3.5. A complete protein recovery (100%) was obtained upon dialysis. The results indicated that formulating IGF-I in 100 mM acetic acid is robust, since IGF-I solubility is high and liquid handling, such as liquid transfer and sterile filtration, is easily performed in this formulation.

In contrast, upon buffer exchange by dialysis, the in-process water formulation became turbid. Some of the protein precipitated during the buffer exchange. IGF-I solubility in the water was significantly lower than the protein prepared in the original his-arg formulation and the acetic acid formulation. The resulted IGF-I concentration, after dialysis into water, was 24 mg/ml. The pH of the water formulation was near neutral at 7.5. Additionally, IGF-I in the water formulation is very sensitive to local pH changes. During sterile filtration, the clear IGF-I in the water formulation often became opalescent upon transferring in glass vials. Apparently, the sodium silicate deposits on the 30 ml glass vials, which could occur during glass cleaning and sterilization, caused local pH changes and induced IGF-I denaturation. Therefore, IGF-I liquid became opalescent at the liquid and the sodium silicate contact sites. Glass vials rinsed with Milli Q before receiving the water formulation would eliminate this problem. Results indicated that IGF-

I can be prepared in water. However, IGF-I solubility is limited to 24 mg/ml and this liquid formulation is sensitive to minor container pH variation.

Chemical stability of IGF-I in liquid formulations for excipient free powders was monitored by SEC-HPLC and reversed phase HPLC. The chromatographic results indicated that IGF-I prepared in the acetic acid and the water formulations was similar to that of the his-arg formulation and IGF-I liquid product (data not shown). Upon storage for 30 days at 2 - 8°C, IGF-I monomer content as measured by sizing HPLC and main peak area as measured by the reversed phase HPLC remained comparable to the liquids tested immediately after buffer exchange. Results suggested that the water and the acetic in-process bulks, formulated for the preparation of excipient free IGF-I aerosol dry powders, can be stored for 30 days at 2 - 8°C before processing into powders.

Two formulations, containing only arginine as the excipient, were prepared. This is an attempt to eliminate the histidine in the his-arg formulation, for the concerns that histidine might convert to histamine and trigger an allergic reaction of the airway. After buffer exchange by dialysis, the 230 mM and 50 mM arginine formulations are clear and the pHs are similar at ~7.2. The resulted IGF-I concentration from the 230 mM arginine formulation was 30.8 mg/ml and a 99.4% protein recovery was obtained. Whereas, the resulted IGF-I from the 50 mM arginine formulation was only 16.1 mg/ml and a 49% recovery was obtained. Approximately 50% of the protein was lost during buffer exchange into the low arginine formulation. IGF-I was not detected in the 50 mM arginine outside the dialysis cassette (data not shown). Results suggested that IGF-I in 50 mM arginine interacted and adhered to the dialysis membrane, therefore resulted in this low protein recovery. These results suggested 50 mM arginine is not a suitable in-process formulation candidate for IGF-I powder preparation, and it was not carried forward in the study.

IGF-I in the 230 mM arginine formulation and the original his-arg formulation was monitored for its chemical stability. Upon storage at 2 - 8°C for 30 days, IGF-I is stable. Results indicated that the 230 mM arginine and the his-arg solution (230 mM arginine

plus 10 mM histidine, pH 7.3) are good formulation candidates for IGF-I aerosol powder preparation.

Two salt containing acidic formulations, 10 mM sodium citrate, pH 5.4 and 100 mM acetic acid plus 100 mM NaCl, pH 3.0 were prepared. Results similar to the formulation containing acetic acid alone were obtained for the acetic acid plus NaCl formulation. High IGF-I concentration (28.1 mg/ml) and complete protein recovery were obtained. The results suggested that this formulation is suitable for preparing IGF-I liquid.

In contrast, upon dialysis into the sodium citrate formulation, IGF-I gelled and adhered to the dialysis membrane. By robbing the membrane, IGF-I formed slimy, white strands. These protein strands adhered tightly to and could not remove from the dialysis membrane. The recovered IGF-I liquid from the dialysis cassette was clear. Results indicated that the dialysis process lost 75% of the original IGF-I to the membrane and only 8.1 mg/ml of IGF-I remained soluble in the sodium citrate formulation. These results suggested that 50 mM sodium citrate at pH 5.4 is not a suitable liquid formulation for IGF-I powder preparation, and it was not carried forward in the rest of the study.

Chemical stability of IGF-I in acetic acid plus NaCl liquid formulation was monitored. Results similar to the formulation using acetic acid alone were obtained. The results indicated 100 mM acetic acid plus 100 mM NaCl, pH 3.0 is a suitable formulation for IGF-I powder preparation. However, the addition of NaCl to the acetic formulation showed no apparent benefit in IGF-I solubility and recovery of the in-process liquid.

The in-process liquid formulation, after storing at 2 - 8°C for 30 days were tested for bio-activity. No apparent loss of IGF-I bioactivity was observed.

Results indicated that the water, the acetic acid with or without NaCl, the 230 mM arginine and the his-arg formulations offered high IGF-I solubility, bioactivity, and acceptable 30 day chemical stability. Therefore these formulations are suitable for the preparation of IGF-I powders.

The stability of IGF-I in the excipient free and the simple formulations of the in-process liquid bulks for aerosol powders were examined after 8 month storage at 2 - 8°C using SEC-HPLC and acidic pH RP-HPLC. Results indicated that a significant loss of IGF-I monomer form was obtained for the water formulation. After 250 days storage at 2 - 8°C, approximately 17 % of IGF-I became aggregated in the water formulation. Significant amount of IGF-I dimmers, oligomers, and higher molecular weight species were detected (data not shown). In addition, approximately 5% of misfolded IGF-I form was also detected. It is possible that the conformation of IGF-I in water is slightly different from the native form and is not very stable, therefore, IGF-I formulated in water alone is more acceptable for aggregation and disulfide isomerization. These result suggested that IGF-I formulated in water needs to be processed quickly (within a month) to prevent significant degradation before the preparation of IGF-I powder.

Chemical stability of IGF-I in the acidic acid containing formulations (acetic acid alone and acetic acid plus NaCl, pH 2.8) was examined after 8 month storage at 2- 8 °C. HPLC results indicated that very little IGF-I was lost to the aggregation reaction and less than 5% of IGF-I was lost to the misfolded and the oxidized IGF-I formation. Additionally, results also suggested that the presence of NaCl slightly reduced IGF-I chemical degradation reactions in the acetic formulation. Results suggested that the acidic acid formulations (with or without NaCl) are excellent formulations for IGF-I powder preparation in terms of in-process liquid stability. The presence of NaCl in the acetic formulation slightly enhanced IGF-I stability. The stability of these formulations would offer excellent flexibility in the manufacturing scheduling of IGF-I powder production.

IGF-I in the arginine containing formulations (230 arginine alone and the his-arg formulation) show significantly more IGF-I aggregates than the protein formulated in the acetic acid containing formulation. Significant amount of misfolded protein was also detected in the arginine containing formulations. These result suggested that IGF-I formulated in the arginine containing formulations need to be processed quickly (within a month) to prevent significant degradation.

The pH of the water formulation and the arginine containing formulations is near neutral (pH 7.3 - 7.5), while the pH of the acetic acid containing formulation is very acidic (pH 2.9 - 3.5). Stability data of these formations suggested that IGF-I liquid is not stable near neutral pH. Neutral pH appeared to promote IGF-I aggregation and the formation of misfolded variant. This is not surprising, since it was reported that disulfide isomerization usually occurs in the alkaline pH and does not occur in an acidic condition.

Overall, these results suggested that acetic acid containing formulations (100 mM acetic acid with or without 100 mM NaCl) are the best in-process liquid formulations for the preparation of IGF-I powders. The presence NaCl could offer a slightly enhanced IGF-I chemical stability. The advantages of the acetic formulations are: 1) high concentration of IGF-I can be obtained in these formulations to minimize the volume and process time for IGF-I dry powder preparation; 2) IGF-I is stable and not sensitive to minor environmental pH changes in these formulations; 3) IGF-I is chemically stable in these formulations to provide excellent manufacturing scheduling flexibility.

Spray Drying Process:

Spray drying was a multi-parameter process and was performed using a mini spray dryer Büchi model 190. The solution was atomized by a two-fluid nozzle (0.5 mm) using compressed air from in-house supply (approximate at 5.5 bars). The air was filtered through a 0.22-μm Milidisk filter (Millipore) before entering the nozzle and the flow rate was controlled by a peristaltic pump (Cole Parmer). Cooling water was circulated through a jacket around the nozzle. Modifications of the original set-up for scale up operation included replacement of the bag-filter unit with a vacuum cleaning unit (Model 005, VAC-U-MAX) and relocation of the aspirator to the drying air input. The operating condition was: inlet temperature of 100°C, aspiration rate of 1,000 L/hr, atomizing air flow rate of 1,050 L/hr, and liquid feed rate of 15 mL/min. This condition resulted in an outlet temperature of 54°C.

Dispersibility measurement

The spray-dried powder was blended with 100M lactose coarse carrier at 1:10 weight ratio of active rhIGF-I to coarse carrier by mixing (Turbula, Glenn Mill) and sieving (250-μm

mesh). Ten individuals of pre-weighted samples of 10 mg blended powder (or 5 mg raw powder) were loaded into a dry powder inhaler (Dura Pharmaceuticals, San Diego) and dispersed into a multi-stage liquid impinger (MSLI) at an air flow rate of 60 L/min and an inhalation time of 5 seconds. The MSLI throat piece was attached to the top of the first stage. A filter paper was placed underneath stage 4 to capture fine particles in range of less than 1 μm. The material which deposited in the throat piece and the filter and their washings analyzed for protein content. The fine particle fraction is defined as powder with an aerodynamic mass median diameter of less than 6.8 μm, and was determined by the percentage of protein which deposited on stages 3, 4 and filter.

Moisture Content:

The residual moisture of the powder sample was determined using a thermogravimetric analyzer (Perkin-Elmer, Model TGA7) in which the sample was heated at a rate of 4 ¡C/min from ambient temperature to 200°C. Percent moisture was calculated as the weight loss between ambient temperature and 150°C where the profile leveled off.

Scanning Electron Microscopy (SEM):

The morphology of the spray-dried rhIGF-I particles was determined by SEM. Samples were sprinkled on SEM stubs and coated with a layer of 10 nm goal-platinum. Scanning electron micrographs were obtained using a Philip SEM system (Model 525M) scanning at the voltage of 5.0 kV.

Particle shape and morphology can be classified into 5 groups among the 12 formulations, represented by (a) "raisin-like" (Form. A, B, C, F, and G) D; (b) "dimpled" (Form. E, H, I, and L); (c) "fused" (Form. K); (d) "fused raisin" (Form. D); (e) "needle crystal" (Form. J), as shown in Figure 2. A previous study (Maa et al., Pharm. Dev. Tech. 2(3):213-223 (1997)) concluded that spray-drying conditions and protein formulations have a strong influence on the morphology and shape of the spray-dried particles based on the "dry-crust" hypothesis. Therefore, the differences in morphology and shape could be attributed to formulation variables because all these powders were prepared under the same drying conditions. The particle of excipient-free rhIGF-I (Formulation A) showed a raisin-like morphology, and the addition of some

excipients such as acetic acid, arginine (pH 7.4), and sodium chloride did not affect the morphology. However, the addition of histidine, trehalose, smoothed out the surface with dimples. If formulated at a low pH (5.5), arginine caused spray-dried particles to fuse together to form a larger particle ("fused") because the formulation became hygroscopic. The current liquid formulation (Form. D) was so complex (4 components) that it resulted in a "fused-raisin" morphology. The particles containing histidine and mannitol (formulation J) showed dimpled particles intermingled with needle-shaped crystals. Mannitol is known to crystallized upon spray drying (see Maa, supra) although it occurred normally at concentrations higher than 70:30 weight ratio of protein to mannitol.

Particle Size Analysis:

The particle size distribution of the powders were determined in liquid suspensions on a laser diffraction instrument (MasterSizer, Malvern). Each powder was first dispersed in isopropanol which has been pre-saturated with the excipients used in the powder was filtered twice through a 0.22-μm filter (Millipore) prior to use. The suspension was sonicated for a minute using an ultrasonic bath (model B3-R, Cole-Palmer). An aliquot of the suspension was loaded to the sample cell. The laser passed perpendicularly through the sample cell and into a lens with a focal length of 100 mm. The intensity of scattered light was measured at different angles, and the results were used to calculate the volume median diameter and the distribution span. The analysis was done in the independent mode with the presentation set at 1400. The volume median diameter is the diameter at 50% of the volume distribution. The span = [D(v, 90)-D(V, 10)]/D(v, 50) where D(v, 90), D(v, 10) and D(v, 50) are the diameters of 90, 10 and 50% cumulative volume, respectively. Each measurements was the average of 3 measurements.

Table I summarizes the particle size and the size distribution (span) of the spray-dried rhIGF-I powders (Form. A - L). All powders in Groups (a), (b), and (e) were smaller than 4 μm in medium diameter and their span was less than 1.5. The fused powder (Form. K) had a largest size (7.3 μm) and a broader size distribution (span=2.3). The fused-raisin powder (Form. D) had a normal size (3.9 μm) but a very broad size

distribution (span=5.2). The moisture content of all powders fell in the range of 5 - 7% and did not correlate with protein formulations.

Table I: Summary of Physical Characterization of rhIGF-I Powders

Formulation	Particle Size [c] (μm)	Span
Spray-dried		
A	4.1	1.3
B	3.5	1.3
C	3.9	1.4
D	3.8	5.2
E	3.7	1.3
F	5.1	4.4 [b]
G	3.6	1.4
H	2.8	1.2
I	2.6	1.2
J	2.8	1.2
K	7.3 [a]	2.3
L	--	--

[a] Measurement was not done immediately off the spray dryer. Powders were stored in focal tubes, and caked out during storage at ambient conditions. Therefore, they were not dispersed well in the dispersant.

[b] There was impurity present in the measurement.

[c] The volume median diameter is the diameter above and below which 50% of the volume distribution lies. The span = [D(v, 90)-D(V, 10)]/D(v, 50) where D(v, 90), D(v, 10) and D(v, 50) are the diameters of 90, 10 and 50% cumulative volume, respectively.

Storage Conditions.

Samples were stored in open glass vials inside sealed desiccators which contained saturated salt solution to control the humidity: calcium chloride at 38% relative humidity (rh). Temperatures were maintained by placing the sealed containers in constant, controlled temperature storage cabinets. Samples of both raw powders and formulated blends were stored at 2-8°C and at 30°C. The powders were assayed for

soluble aggregates, oxidation, and aerosol performance at t = 0, 4 weeks, and 24 weeks of storage.

The chemical and physical stability of IGF-I powders were determined by the following standard assays: pH, acidic pH reversed phase HPLC chromatography to quantitative the amount of oxidized and clipped variants and native size exclusion chromatography (SEC-HPLC) to determine the amount of product aggregation. IGF concentration and presence of excipient(s) were also determined by SEC-HPLC. IGF-I activity was determined by the bioassay that measures the effect of IGF-I in promoting H3 thymidine uptake in BALB3T3 cells.

The amount of main peak remaining was calculated as an area percentage of the total IGF-I area of each chromatogram (e.g., % main peak for RP-HPLC = area counts of main peak/total protein area counts from RP-HPLC * 100). The reaction rate constant (k) of each degradation was obtained from the linear regression line fitted through the data of log (% main peak area), from HPLC analysis, versus time in days. The slope value of the straight line fit was used as the reaction rate constant (k). That is:

$$\log(\%\ \text{main peak area}) = \log(\%\ t0) - k * \text{days}$$

Where t0 is the main peak amount for the HPLC assay at time zero. In the case of aggregation and benzyl alcohol concentration, the data are presented with percentage monomer remaining and with percentage benzyl alcohol remaining, respectively, at each time point.

IGF-I lots C9845AX were used as the control sample for the chemical stability study.

The effect of spray drying on protein aggregation and oxidation was investigated by size exclusion and reverse phase HPLC. The results suggest that protein quality in all formulations except Form. D remained unchanged upon spray drying. rhIGF-I in Form. D suffered approximately 2% of aggregation; therefore, the further investigation on this formulation was discontinued.

Aerosol Performance (Fine Particle Fraction)

The spray-dried powder was blended with 100 M lactose carriers prior to fine particle fraction (< 6.8 μm) measurement using a multi-stage liquid impinger model. Blending can theoretically improve the fine powder's flow properties. Small particles tend to interact with themselves (agglomeration) and with any contact surfaces due to high surface energy. Agglomerated particles behave like large particles and are difficult to be dispersed. Sticking to other contact surfaces results in material loss and poor powder flowability. If the interaction between the spray-dried particle (raw powder) and the carrier particle (F_{r-c}) overcomes the interaction among the raw powder (F_{r-r}), it can result in homogeneous blending, thereby enhancing the powder's flowability. The next hurdle to jump is that these fine particles should be able to be deagglomerated from the carrier particle upon inhalation, i.e. the inhalation force can overcome F_{r-c}. Factors affecting these interactions are highly complicated.

At t = 0 (before storage), the fine particle fraction of the powder was highly depending upon protein formulation, ranging from 12% to 76% (Table II). Excipient-free rhIGF-I (Form.A) showed the highest FPF, suggesting that rhIGF-I itself was highly dispersible. The addition of other excipients to rhIGF-I may be necessary for the benefit of protein stability, particularly over long-term storage. However, these excipients might change the surface properties of the particles and deteriorate rhIGF-I's excellent dispersibility. Interestingly, the rhIGF-I's dispersibility remained unchanged upon the addition of acetic acid and sodium chloride (Form. B & C). The powder containing 10 mM histidine at pH 5.5 (Form. E) became much less dispersible (FPF = 24%). Adding a high concentration (230 mM) of arginine at pH 7.4 (Form. F & G) caused a decrease in dispersibility but the FPF remained acceptable (close to 50%). The addition of trehalose and mannitol into the histidine-containing formulation significantly reduced the powder's aerosol performance with the FPF being barely over 20% (Form. H & I). A further increase in trehalose concentration (Form. J) dropped the FPF below 20%. In view of the importance that sugar excipients are the common protein stabilizers, it will make aerosol powder development difficult if a high concentration of the carbohydrate excipient is needed for protein stabilization. It is not surprising that the powder of Form. K was least dispersible (FPF = 12%) because of its large particle size. Although being identical to Form. K except a higher pH (7.4), the powder of Form. L was less hygroscopic and possessed a decent dispersibility (FPF = 52%).

Table II: Summary of Physical Properties with Respect to Fine Particle Fraction of rhIGF-I Blends/Raws Stored at 38% RH

Formulation	t = 0	Fine Particle Fraction (%)			
		t ≤ 4 weeks		t ≥ 24 weeks	
		2 - 8 °C	30 °C	2 - 8 °C	30°C
Spray-dried					
A	76	92	76	71	76
B	75	77	81	76	68
C	74	81	71	67	67
D	ND				
E	24	25	25	26	31
F	41	26	51	20	48
G	52	caked	50 [44 [a]]	caked	53 [43]
H	21	clumpy	--	clumpy	--
I	23	20	19.0	caked	--
J	16	--	--	--	--
K	12	--	--	--	--
L	52	28	43	26	49 [47]

[a] Dispersion data of raw powder.
ND Not determined.
clumpy--several particles agglomerated or fused together as a big particle.
caked--particles formed a cake in a vial.

All powders with a high FPF (> 40%) showed a homogenous blending (Figures 2a-c). Nonhomogeneous blending (Figure 2d for Form. H), where the spray-dried particles were highly cohesive ($F_{r\text{-}r} > F_{r\text{-}c}$), showed a poor dispersibility. This demonstrates that the aerosol performance of some powder formulations was dominated by the raw powder's cohesive force ($F_{r\text{-}r}$).

Effect of Long-Term Storage on FPF.

All rhIGF-I blends showing a FPF > 20% at t = 0 were stored at 2-8°C (48% rh) and 30°C (38% rh), and their aerosol performance was determined after 4 and 24 weeks storage (Table II). Powders from Form. A - C remained highly dispersible at 24 weeks regardless

of storage conditions. Storage conditions did not affect the aerosol performance of the Form. E powder either. Other powders (Form. F - I, and L) either suffered a dramatic decrease in FPF or caked up and failed to be dispersed at the 2-8 °C storage. However, powders (Form. F, G, & L) maintained their dispersibility at 30 °C storage. At high rhs (48%), powder formulations containing arginine tended to fuse not only among spray-dried particles but also with the carrier particle (Figure 3 for Form. F after 4-week storage at 2-8 °C). These modifications resulted in significantly reduced aerosol performance, which was confirmed by the particle size analysis on the liquid impinger for Form. F stored at 2-8 °C. The fraction of the powder deposited on Stage 1 (10 - 20 μm) increased significantly with the storage time, but the fractions on Stages 3, 4, & filter decreased with time (Figure 4). Under the same condition, the size distribution for the powder (Form. B) on different components of the impinger remained unchanged over the time.

Some non-blended (raw) powder formulations (G & L) were dispersed after being stored at 30 °C (data shown in the brackets in Table II). Their FPFs were 5 - 20% lower than those of the blended powders, suggesting that overcoming the force of F_{r-r} by blending was not that critical for some spray-dried rhIGF-I powder formulations. This offers us the flexibility of selecting dry powder inhalers which are designed specifically for aerosolizing blended or non-blended powders.

Chemical Stability of Protein Dry Powders

The chemical and physical stability of IGF-I powders were determined by the following standard assays: pH, acidic pH reversed phase HPLC chromatography to quantitative the amount of oxidized and clipped variants (AIGF:32) and native size exclusion chromatography (SEC-HPLC, AIGF:33) to determine the amount of product aggregation. IGF concentration and presence of excipient(s) were also determined by SEC-HPLC. IGF-I activity was determined by the bioassay that measures the effect of IGF-I in promoting H3 thymidine uptake in BALB3T3 cells IGF-I.

Excipient free IGF-I dry powders

The effect of spray drying process on the chemical property of IGF-I in the excipient free formulations were examined immediately after powder preparation. The chromatographic

patterns from SEC-HPLC and acidic pH RP-HPLC were similar for these formulations and were comparable to the product in the liquid formulation for subcutaneous injection (data not shown). These results also indicated that the dry powder preparation procedure did not adversely effect the chemical property of the IGF-I powders prepared from the water and the 100 mM acetic acid formulations.

Although the presence of acetic acid was detected by the olfactory sense, the acid was not detected by HPLC (data not shown). This results suggested that the majority of the acetic acid was evaporated during the dry powder preparation. Therefore, IGF-I prepared from the acetic formulation is practically excipient free. The pH of the IGF-I solutions (~ 10 mg/ml) prepared from the powders is 7.1 and 4.1, for the water and the acetic acid formulations, respectively.

Stability results obtained after storing IGF-I powders at 2 - 8°C for 30 days and 252 days revealed that the rates of IGF-I aggregation and oxidation reactions and the formation of the misfolded and other variants in the excipient free IGF-I powders were comparable to these of the IGF-I liquid product (the control, C9845AX). Degradation rates of IGF-I in the excipient free formulations were calculated and summarized in. Interestingly, IGF-I powders stored at 30°C exhibited a similar chemical stability as the powders stored at the lower temperature (5°C). In addition, the chemical degradation rates of the protein powder were significantly lower than the rates for C9845AX stored at 5°C. This is expected, since protein stored in a dry powder form, in general, is more stable than the protein in liquid formulations.

To test whether the excipient free IGF-I powders render desirable physical property for aerosol drug administration, the dispersibility of protein dry powders was analyzed. Results indicated that regardless of the storage condition (2- 8°C or 30°C), relative humidity (28% or 46%), and duration (day 0 to 8 month), approximately 70 % of the powders are in the fine respirable particle fraction. These results suggested that the water and the acetic formulations are suitable for the preparation of IGF-I dry powders. Furthermore, these results indicated that protein stabilizing reagent is not required for IGF-I dry powder preparation. The fine particle fraction in these excipient free IGF-I powders are stable for at least 8 months at 5°C and 30°C. These excipient free IGF-I powders are not sensitive to the storage condition.

Protein powders prepared from simple formulations

These powders were prepared from the 230 mM arginine and the acetic acid plus NaCl formulations. Chemical stability data revealed that IGF-I powders prepared from simple formulations are stable and the stability is similar to the powders prepared from excipient free formulations. These results also suggested that stabilizing reagent, such as carbohydrates, is not required for the protein dry powder preparation. IGF-I powders from these simple formulations are chemically stable for at least 8 months upon storage. Similar to the excipient free IGF-I dry powders, room temperature storage of these powders appear to be feasible.

Physical analysis results of these simple formulations revealed that the powder prepared from the acetic acid plus NaCl formulation is similar to the excipient free powder prepared from 100 mM acetic acid without NaCl. More than 65% of the powder is in the fine respirable particle fraction. Results indicated that the addition of NaCl showed no apparent effect on the chemical and physical property of the IGF-I dry powders prepared from formulations containing acetic acid.

In contrast, IGF-I powders produced from the 230 mM arginine formulation was 43% respirable immediately upon preparation. This fine particle fraction is only 70 % that of the excipient free and the acetic acid plus NaCl formulations. Furthermore, the powder prepared from the arginine formulation is physically unstable and showed a dramatically reduced fine particle fractions upon storage at 2 - 8°C and ~48% RH. The protein powders formed clumps are not dispersible for aerosol delivery. This poor physical property could be caused by the effect of a higher humidity at the lower storage temperature on arginine. Interestingly, the formation of protein clumps did not affect the chemical stability of the powder.

Protein powders prepared in histidine derived formulations

The histidine derived formulations uses 10 mM histidine at pH 5.5 as the buffering reagent. The effect of carbohydrate, trehalose at 80/20 or 60/40 of IGF-I/trehalose, on the histidine formulation was examined.

However, after storing the powders at 30°C and 27% RH for 30 days, acidic pH RP-HPLC results suggested that the presence of trehalose increased the chemical stability of these IGF-I

powders. The degraded IGF-I level is significantly higher than that obtained from the excipient free powders.

Protein powders prepared from IGF-I liquid final product formulation

IGF-I in the formulation designed for subcutaneous injection was processed to form IGF-I dry powder. Results indicated that the dry powder preparation significantly increased IGF-I aggregate formation. Compared to the initial liquid, IGF-I main peak assayed by the acidic RP-HPLC reduced 99.4 to 98 % (data not shown), while protein aggregation increase 2 - 6 % . These high aggregation levels were not surprising, since the IGF-I liquid product formulation contains 0.9 % benzyl alcohol. During dry powder preparation, the concentration of benzyl alcohol increased and could result in a significant amount of protein denaturation. Powders prepared from the his-arg formulation was also examined. Similar to the arginine alone formulation, the chemical stability of the IGF-I powder prepared from the his-arg formulation is very stable upon storage at 2 - 8°C and ~46% RH or 30°C and ~ 28% RH as assayed by SEC-HPLC for aggregation reaction or acidic pH RP-HPLC or oxidation and misfolded variant formation. The formation of a his-arg containing protein cake upon storage at the lower temperature and the higher relative humidity did not exhibit an apparent effect on the chemical stability of IGF-I.

CLAIMS

We claim:

1. A spray-dried insulin-like growth factor I (IGF-I) dry powder composition for pulmonary administration comprising particles of IGF-I of an average size 2 to 4 μm.

2. A composition according to claim 1 wherein said composition is substantially free of an excipient.

3. A method of preparing a composition according to claim 1 comprising spray-drying an aqueous mixture of the IGF-I under conditions to provide a respirable dry powder.

4. A method for aerosolizing a spray-dried IGF-I dispersible dry powder composition comprising dispersing an amount of the dry powder in a gas stream to form an aerosol.

5. A method according to claim 4 further comprising capturing the aerosol in a chamber suitable for subsequent inhalation by a patient.

6. A method of administering a therapeutically effective dose of IGF-I to a patient comprising administering to the alveolar regions of the lungs of said patient a composition according to claim 1.

7. A method of treating an IGF-I related disorder comprising administering to the alveolar regions of the lungs of a patient a composition according to claim 1.

8. A unit dosage receptacle comprising a therapeutically effective amount of the composition of claim 1.

9. A dry powder inhaler comprising the composition of claim 1.

FIG._1A

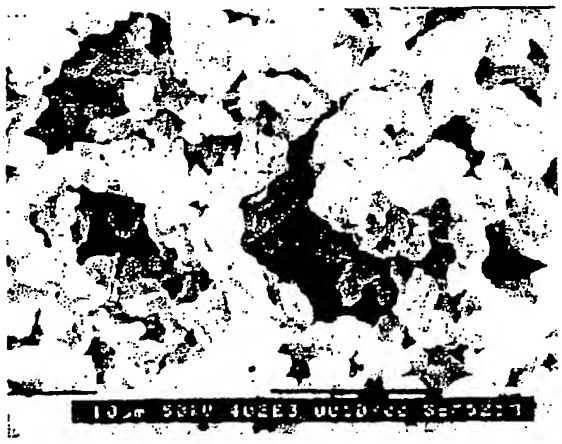

FIG._1D

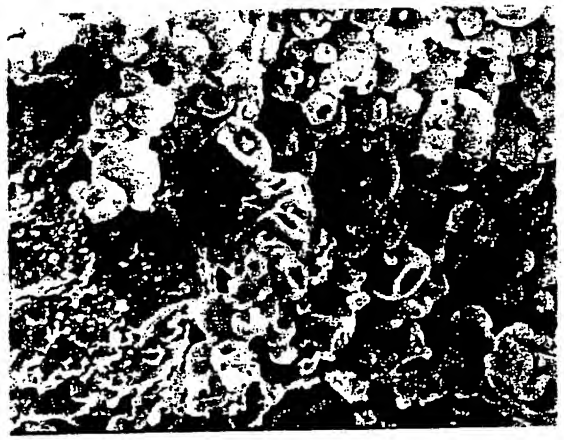

FIG._1B

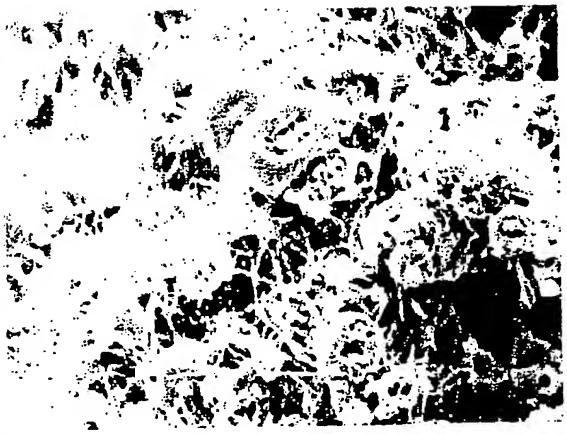

FIG._1E

FIG._1C

FIG._2A

FIG._2C

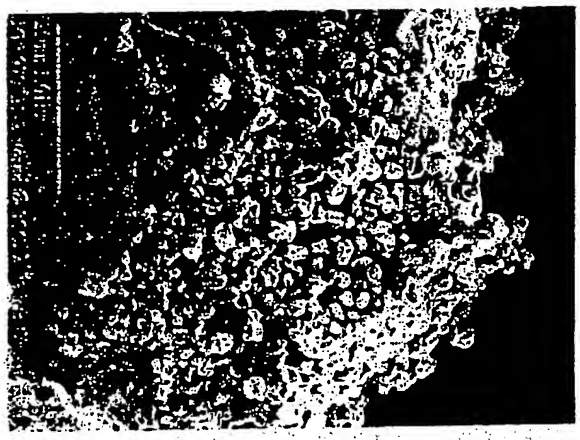

FIG._2B

FIG._2D

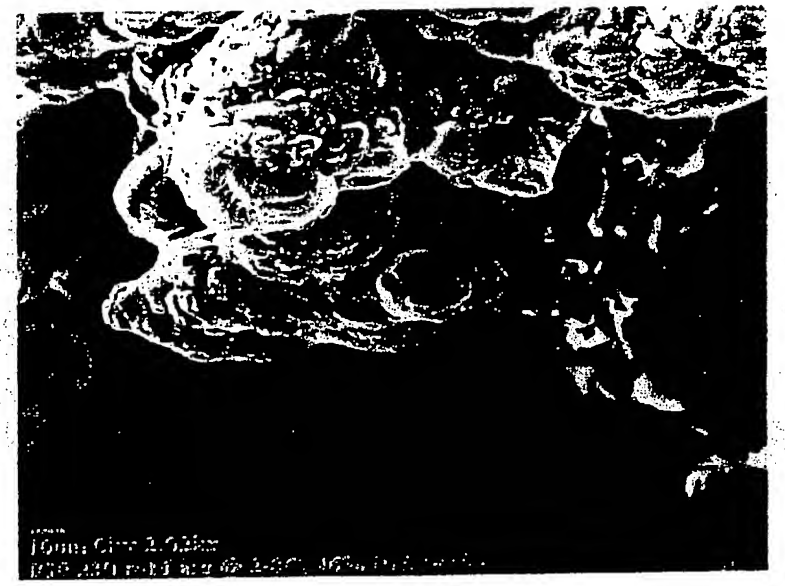

FIG._3A

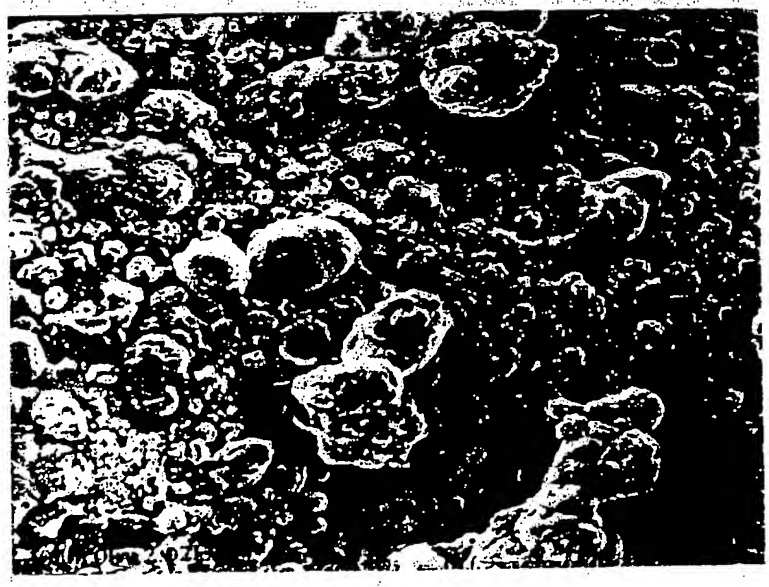

FIG._3B

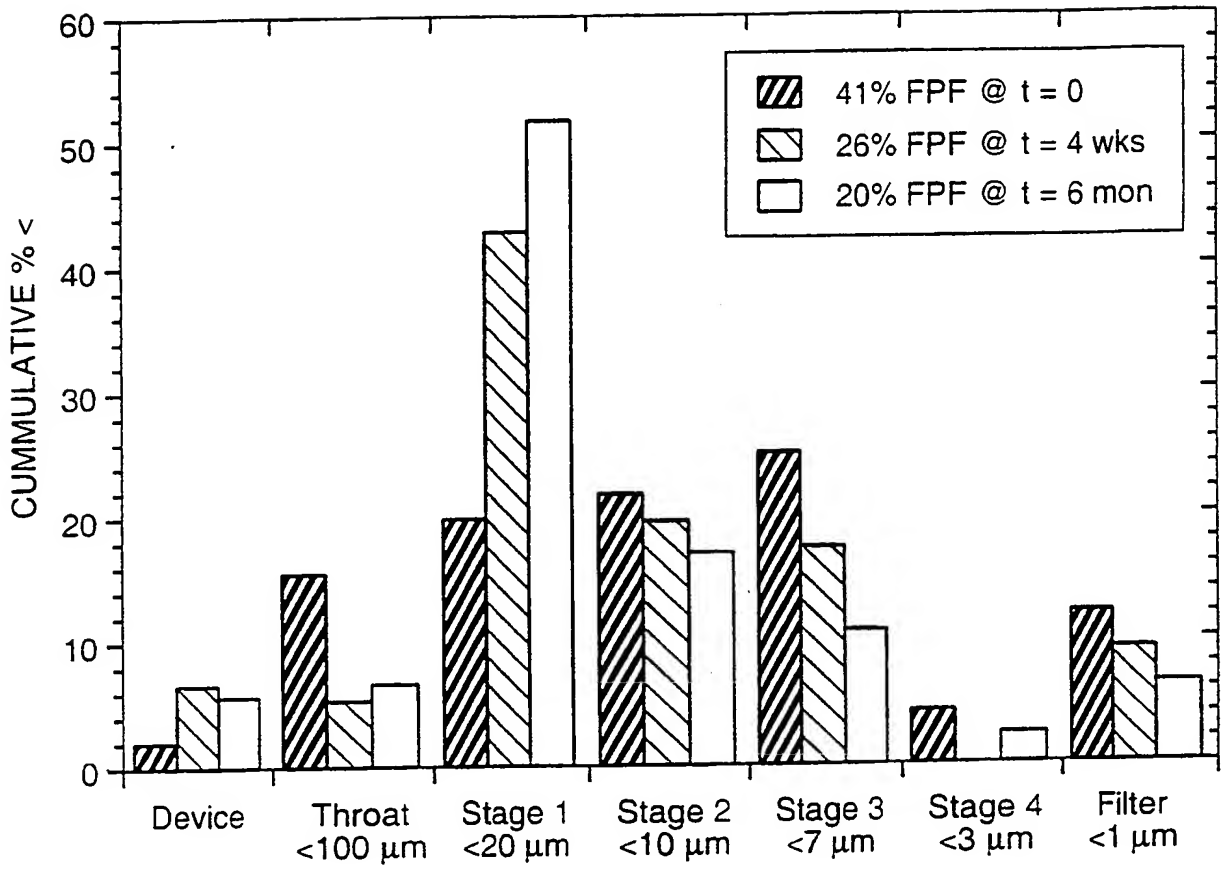
60
50
40
30
20
10
0
CUMMULATIVE % <
41% FPF @ t = 0
26% FPF @ t = 4 wks
20% FPF @ t = 6 mon
Device
Throat <100 µm
Stage 1 <20 µm
Stage 2 <10 µm
Stage 3 <7 µm
Stage 4 <3 µm
Filter <1 µm

FIG._4

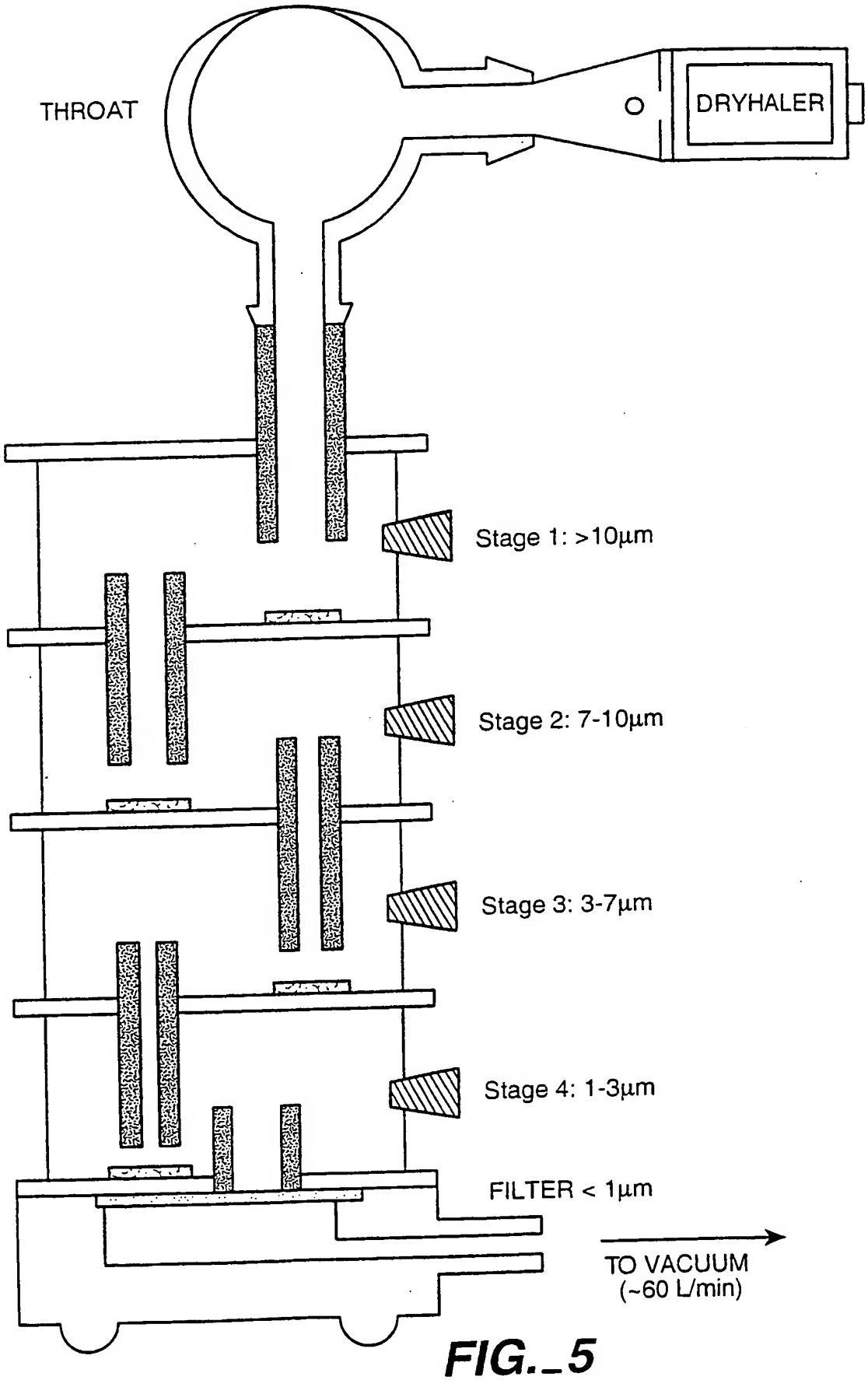
THROAT
DRYHALER
Stage 1: >10μm
Stage 2: 7-10μm
Stage 3: 3-7μm
Stage 4: 1-3μm
FILTER < 1μm
TO VACUUM
(~60 L/min)

FIG._5

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/09077

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/30 A61K9/00 A61K9/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 32149 A (INHALE THERAPEUTIC SYST) 17 October 1996 (1996-10-17) page 1, line 12-15 page 3, line 5-18 page 4, line 8-25 page 9, line 33 - page 12, line 21 page 13, line 30 claims 1,3,5-7,9-12,17-19 ---	1-4,6,8, 9
X	WO 97 41833 A (INHALE THERAPEUTIC SYST) 13 November 1997 (1997-11-13) cited in the application page 9, line 21 - page 10, line 2 page 12, line 29 page 18, line 3-13 claims 1,2,4,14,16,23-25 --- -/--	1,3,4,6, 8,9

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 September 1999	15/09/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	La Gaetana, R

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IN ...RNATIONAL SEARCH REPORT

International Application No
PCT/US 99/09077

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 440 989 A (FUJISAWA PHARMACEUTICAL CO) 14 August 1991 (1991-08-14) page 2, line 3,4 page 2, line 30-52 claim 1	1,2
A,P	MAA YF, NGUYEN PA, ANDYA JD, DASOVICH N, SWEENEY TD, SHIRE SJ, HSU CC: "Effect of spray drying and subsequent processing conditions on residual moisture content and physical/biochemical stability of protein inhalation powders" PHARMACEUTICAL RESEARCH, vol. 15, no. 5, May 1998 (1998-05), pages 768-775, XP002114088 cited in the application abstract page 769, paragraph "Protein powder preparation"	1

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/US 99/09077

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 7 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Information on patent family members

International Application No
PCT/US 99/09077

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		JP 4208228 A	29-07-1992
		JP 8018999 B	28-02-1996
		US 5210074 A	11-05-1993